

Figure 10A-10N of the application as filed. Support for new SEQ ID Nos. 48-53 is to be found *inter alia* at page 43, lines 14-21, of the application as filed.

A copy of the Sequence Listing in computer-readable form is submitted herewith, as required. The undersigned states that the paper copy and the computer-readable form of the Sequence Listing are identical.

Conclusion

It is believed that the present submission does not require the payment of any fees under 37 C.F.R. 1.16-1.17. If this is incorrect, please charge any fee due under the foregoing Rules to Deposit Account No. 07-1969.

Respectfully submitted



Donna M. Ferber
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Suite 201
Boulder, CO 80303
Telephone (303) 499-8080
Facsimile: (303) 499-8089
Email: winner@greenwin.com

Attorney docket No. 64-99
bmk: 11 January, 2001

- 10 -

derived from several isolates of *P. falciparum* (Pf) [i.e. Pf(C10), SEQ ID NO: 23; Pf10/P, SEQ ID NO: 24; Pf11/P, SEQ ID NO: 25; Pf19/I, SEQ ID NO: 26; Pf20/L, SEQ ID NO: 27; and Pf18/S, SEQ ID NO: 28], *P. vivax* (Pv) [i.e. Pv12/P, SEQ ID NO: 29; Pv13/P, SEQ ID NO: 30; Pv15/I, SEQ ID NO: 31; Pv16/L, SEQ ID NO: 32; Pv17/S, SEQ ID NO: 33; and Pv86/C, SEQ ID NO: 34], *P. malariae* (Pm) [i.e. Pm1/S, SEQ ID NO: 35; and Pm38/S, SEQ ID NO: 38], *P. Ovale* (Po) [i.e. Po35/S, SEQ ID NO: 37; and Po36/S, SEQ ID NO: 38] and *P. berghei* (Pb) [i.e. Pb(ANKA), SEQ ID NO: 39]. The alphanumeric designation following the *Plasmodium* species descriptor indicates the isolate number and geographical origin of the specimen, wherein P=Pakistan, I=India, L=Laos, C=Columbia and S=Singapore. The GenBank accession numbers for Pf(C10) (SEQ ID NO: 23) and Pb(ANKA) (SEQ ID NO: 39) are X95275 and U79731 respectively.

Figure 10A-10N depict a schematic representation of the aligned cox I sequences from *P. falciparum* (Pf) [i.e. PfcoxI, SEQ ID NO: 40; and Pf47coxI, SEQ ID NO: 41], *P. vivax* (Pv) [i.e. Pv15coxI, SEQ ID NO: 42; Pv16coxI, SEQ ID NO: 43; Pv32coxI, SEQ ID NO: 44; and Pv37coxI, SEQ ID NO: 45], *P. malariae* (Pm) [i.e. Pm58coxI, SEQ ID NO: 47] and *P. Ovale* (Po) [i.e. Po35coxI, SEQ ID NO: 46] isolates. The numeric designation following the *Plasmodium* species descriptor indicates the isolate number. The GenBank accession number for the *P. falciparum* sequences is M76611.

Figure 11 is a copy of a photographic representation showing PCR amplification of blood spots. Each reaction uses 1 μ l of blood containing different quantity of parasites. The amount of DNA used in each reaction, expressed as an equivalent number of parasites, is as follows: lane 1 contains 800 parasites; lane 2 contains 400 parasites; lane 3 contains 80 parasites; lane 4 contains 40 parasites; lane 5 contains 8 parasites; lane 6 contains 4 parasites; and lane 7 contains 0.8 parasites. Lane 8 contains the 100bp DNA ladder (Promega) used as a marker. The detection limit is 4 parasites.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In work leading up to the present invention, the inventors have discovered that the molecular composition, physical arrangements and nucleotide sequences of the extrachromosomal plastid-like element and mitochondrial element are highly conserved in different *Plasmodium ssp.*

The inventors have utilised the high degree of homology between different *Plasmodium ssp.* in the design of reliable, genera-specific or species-specific diagnostic assays for the detection of *Plasmodium*. The diagnostic assays described herein provide a significant advantage over

units of Taq DNA polymerase (Amersham) and 0.2 mM of each dNTPs. Reaction tubes were overlaid with one drop of mineral oil. The reaction was soaked at 95°C for 5 minutes then held at 80°C prior to the addition of Taq DNA polymerase and dNTPs. Amplification involved 40 cycles of 1 minute denaturation at 90°C, 2 minute annealing at 52°C and 3 minutes primer extension at 72°C. A 5 minute primer extension at 72°C was included following the final cycle.

3. *Sequences of primers.*

The primers used for amplifying the LSU-rRNA gene were as follows:

- 10 L1 5' GAC CTG CAT GAA AGA TG 3' (SEQ ID NO: 5); and
L2 5'GTA TCG CTT TAA TAG GCG 3' (SEQ ID NO: 6).

A second set of primers were designed to amplify the dihydrofolate reductase-thymidylate synthase (DHFR-TS) gene from *P.berghei* genomic DNA in control experiments:

- 15 DHFR1 5' GCA ATA TGT GCA TGT TGT AAA 3' (SEQ ID NO: 48); and
DHFR2 5'ATT CTT TAT AAA CAG ACG 3' (SEQ ID NO: 49).

The primers used for amplifying the human β -actin gene were as follows:

- AC1 5' GGG CGA CGA GGC CCA GAG C 3' (SEQ ID NO: 50);
20 AC2 5' GCA TCC TGT CGG CAA TGC C 3' (SEQ ID NO: 51);
AC3 5' AAG GAG AAG CTG TGC TAC 3' (SEQ ID NO: 52); and
AC4 5' TCA TGA TGG AGT TGA AG 3' (SEQ ID NO: 53).

4. *Agarose gel electrophoresis*

- 25 10 μ l of each PCR product was resolved in 1% agarose gels with TAE electrophoresis buffer (40mM Tris-acetate and 1mM EDTA, pH 8.0). Electrophoresis was carried out at 100V for 1.5 hours and the fragments were visualized under UV.

5. *DNA sequencing protocol*

- 30 The PCR products were loaded onto a 1% (w/v) low-melting point agarose gel and extracted